MODERATE AND HIGH AMOUNTS OF PROTEIN FROM CASEIN ENHANCE HUMAN ABSORPTION OF ZINC FROM WHOLE-WHEAT OR WHITE ROLLS

Janet R. Hunt, Ph.D.,1 Glenn I. Lykken, Ph.D., and Loanne K. Mullen, R.D.
United States Department of Agriculture, Agricultural Research Service
Grand Forks Human Nutrition Research Center
Grand Forks, ND 58202

ABSTRACT

The enhancement of zinc absorption by protein was investigated using a broad range of ingested protein in combination with either whole-wheat or white rolls. Twenty or 40 g of protein from casein were added to rolls containing 10 g protein; zinc content was adjusted to 4 mg. The rolls were extrinsically labelled with 65Zn and fed to 20 healthy participants. Zinc absorption was measured by whole body scintillation counting. Average (± SD) zinc absorption ranged from 8 ± 4% for the whole-wheat rolls with the least protein to 26 ± 7% for the white rolls with the most protein. Less zinc was absorbed from whole-wheat than from white rolls containing the same amount of zinc. The protein and type of flour did not interact to affect zinc absorption. As protein increased from 10 to 50 g, zinc absorption was enhanced with no indication that maximum enhancement had been achieved.

KEY WORDS: zinc absorption, protein, casein, bioavailability

INTRODUCTION

Zinc absorption from foods is influenced by the amount of zinc consumed and the presence of other dietary components including protein and phytic acid (1). Increased protein content in meals has been associated with increased zinc retention in both human (2,3) and animal (4,5) studies. Sandström et al. (2), using meals supplemented to control zinc content, found a positive, linear association between zinc absorption and the protein content of meals containing 5 to 27 g of protein. In that study, each level of protein was from a different protein source; thus, protein was not the only variable. The addition of cheese and milk appeared to enhance zinc absorption to a greater extent with whole-meal bread (enhancement from 8.2 to 14.0%) than with white bread (enhancement from 13.2 to 15.7%) (2). Studies with rats also indicated that zinc absorption was positively associated with the protein content of meals containing various protein sources (4) and that the amount of protein needed to enhance zinc absorption depended on the amount of zinc in the meal (5). When low amounts of zinc were consumed, the enhancing effect of protein on zinc absorption appeared to reach a maximum beyond which additional protein had less effect (5).

The purpose of the present investigation was to determine the effect of the addition of 20 or 40 g protein from a single source on zinc absorption from whole-wheat or white rolls. Casein was chosen as a protein source to allow comparisons to the study by Sandström et al. (2), which utilized various combinations of milk, cheese, and casein, in addition to egg and beef. Zinc content of the rolls was controlled to allow examination of the effects of protein level and flour type independent of the zinc content. It was hypothesized that protein, by enhancing zinc solubility in the intestine, would counteract the inhibiting effects of phytic acid and fiber on zinc absorption.

1Reprints: JR Hunt, Ph.D., USDA-ARS Human Nutrition Research Center,
PO Box 7166, University Station, Grand Forks, ND 58202, USA
resulting in greater enhancement of zinc absorption when protein was added to whole-wheat rather than white rolls. In addition, we wanted to determine whether the effect of protein on zinc retention was linear between 10 and 50 g protein, or whether zinc retention would reach a maximum beyond which additional protein had little effect.

**METHODS AND MATERIALS**

**Subjects** - This research with human subjects was approved by the USDA Human Studies Review Committee and by the University of North Dakota Radioactive Drug Research Committee and Institutional Review Board. Twenty healthy adults (5 men and 15 women) who were recruited locally consented to participate. They ranged in age from 19 to 69 years, with a mean (± SD) height of 168 ± 11 cm and weight of 79 ± 21 kg. Three times, at approximately seven-week intervals, participants consumed $^{65}$Zn-labelled rolls at the Research Center. Three-day food records and fasting blood samples were obtained each time.

$^{65}$Zn-labelled rolls - Three levels of protein were fed to each volunteer in rolls: half of the participants consumed only rolls made with whole-wheat flour and the other half consumed only rolls made with white flour. The type of flour and the order of feeding the different protein levels were randomly assigned, with the restriction that a similar number of males and females were fed each type of wheat flour.

Each serving of rolls included 0, 22, or 44 g sodium caseinate (Ultra Supreme Grade, Erie Casein Co., Inc., Erie, IL), 60 g stone ground whole-wheat (100% extraction) or enriched, all-purpose white flour (both Occident brand, Peavey Co., Minneapolis, MN), 25 g butter, 1 g salt, 20 g grape jelly, and 240 mL deionized water. The zinc content of the rolls was adjusted with zinc sulfate to 4.0 mg, the amount in the whole-wheat rolls with 44 g added sodium caseinate. The addition of 22 and 44 g sodium caseinate provided 20 and 40 g protein, respectively, supplementing the 9-10 g protein provided by other ingredients (mainly wheat). The phosphorus content of the rolls was allowed to vary with the sodium caseinate and flour type. Rolls with whole-wheat flour contained 652 μmol (430 mg) phytic acid (by analysis) and a phytate to zinc molar ratio of 11. Phytic acid was not detectable in rolls containing white flour. Each serving contained 3700 Bq (0.1 μCi) $^{65}$ZnCl$_2$ (Dupont New England Nuclear, Boston, MA) injected into the baked rolls at several distinct sites.

The rolls were consumed in the morning preceded by a 12-hr fast and followed by a 4-hr fast, a sack lunch low in zinc (1.4 mg), and another 4 hr with food intake limited to coffee, tea, or carbonated beverages.

**Measurement of zinc absorption** - Retention of $^{65}$Zn was measured using a whole body counter equipped with 32 thallium-doped sodium iodide crystals (each 4 x 4 x 16 inches) arranged in two planar arrays, one above and one below the subject, who was counted in a supine position. Counting measurements were taken the day of the meal and weekly during the following seven weeks. Percent retention was calculated by extrapolating back to the time of the meal along the linear portion of a semilogarithmic retention plot (the natural logarithm of percent remaining radioactivity versus time) (6). Corrections were made for differences in body size and radioisotope distribution (7), physical decay, and residual activity remaining from isotope administrations. Zinc retention determined by this method presumably represents the zinc from the rolls which was absorbed and incorporated into body zinc pools with gradual turnover.

**Estimation of zinc intake from food records** - Three-day food records completed by participants were reviewed with a nutritionist and coded for computation of zinc intake using the GRAND nutrient analysis system. The database for this system utilized USDA (8) and other published data (9,10,11).

**Chemical analyses** - The zinc content of each serving of rolls was determined by inductively coupled plasma emission spectrophotometry after digestion of aliquots with concentrated nitric and 70% perchloric acids by method (II) A of the Analytical Methods Committee (12). Zinc measurements were 97.6% of certified values for zinc in bovine liver samples from the U.S. National Institute of Standards and Technology. Plasma zinc concentrations were determined by flame atomic absorption spectrophotometry after dilution with deionized water, and using standards containing 5% glycerol (13). Phytic acid in the rolls was determined as phytate phosphorus (4), assuming a phosphorus/phytate molar ratio of 6. Protein content was determined by a micro-Kjeldahl analysis of nitrogen (14) using a conversion factor of 6.25 g protein per g nitrogen.
Statistical analyses - The effects of protein level, flour type, and their interaction were tested by analysis of variance; Bonferroni contrasts (15) were used to compare the effect of flour type within each protein level, and the effect of protein level within each flour type. The model accounted for differences between individuals within each flour type consumed: each person served as his or her own control. Actual absorption values are reported without "standardization" to any reference meal. The influence of plasma zinc or of dietary zinc before the rolls were served was examined by correlation and multiple regression analysis (15).

RESULTS

Both the protein content and type of flour significantly affected zinc absorption (Table 1). There was no detectable interaction between the type of flour and the amount of protein in the rolls, although the differences between whole-wheat and white flour seemed somewhat greater at lower protein levels. Zinc absorption ranged from 2 to 36% for individual volunteers across all treatments. The mean absorption ranged from 8% for the whole-wheat rolls with the least protein to 26% for the white rolls with the most protein. For each type of flour, zinc absorption was significantly lower from the lowest and intermediate protein levels than from the highest protein level. Zinc absorption was not significantly different between the two types of flour at specific protein levels, but was different when all protein levels were considered together.

There was no indication of a maximum effect of protein at the higher protein levels. The protein content of the rolls explained 51% of the variation in zinc absorption by linear regression analysis. Nonlinear models could not explain the variance in the data better than the linear model.

Zinc absorption was not associated with either plasma zinc or zinc intake for one or three days before eating the rolls. Plasma zinc ranged from 9.6 to 16.2 μmol/L, which is similar to the normal range of 9.9 to 17.6 experienced at this laboratory. Average calculated zinc intake for three days ranged from 2 to 23 mg/day. Dietary zinc for three days before eating the rolls was positively associated with zinc absorption in a multiple regression model that included protein and flour type as predictive variables. However, this statistical relationship became nonsignificant if a single, high value for zinc intake was omitted from the data set. Therefore, we concluded that dietary zinc for three days before eating the rolls did not affect zinc absorption.

<table>
<thead>
<tr>
<th>Protein g†</th>
<th>Flour Type</th>
<th>Phosphorus mg†</th>
<th>Food Zn mg‡</th>
<th>Added Zn mg</th>
<th>Total Zn mg</th>
<th>Zn Absorbed %</th>
<th>Zn Absorbed mg†</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.9</td>
<td>White</td>
<td>90 ± 6</td>
<td>0.62 ± 0.05∗</td>
<td>3.5</td>
<td>4.1</td>
<td>13 ± 2∗</td>
<td>0.5 ± 0.1∗</td>
</tr>
<tr>
<td>29.6</td>
<td>White</td>
<td>256 ± 20</td>
<td>1.44 ± 0.05</td>
<td>2.5</td>
<td>3.9</td>
<td>18 ± 7</td>
<td>0.7 ± 0.3</td>
</tr>
<tr>
<td>50.5</td>
<td>White</td>
<td>461 ± 28</td>
<td>2.44 ± 0.13</td>
<td>1.5</td>
<td>3.9</td>
<td>26 ± 7</td>
<td>1.0 ± 0.3</td>
</tr>
<tr>
<td>10.6</td>
<td>Whole-Wheat</td>
<td>221 ± 10</td>
<td>1.90 ± 0.08</td>
<td>2.0</td>
<td>3.9</td>
<td>8 ± 4</td>
<td>0.3 ± 0.2</td>
</tr>
<tr>
<td>30.8</td>
<td>Whole-Wheat</td>
<td>396 ± 47</td>
<td>2.76 ± 0.23</td>
<td>1.0</td>
<td>3.8</td>
<td>14 ± 5</td>
<td>0.5 ± 0.2</td>
</tr>
<tr>
<td>51.7</td>
<td>Whole-Wheat</td>
<td>616 ± 24</td>
<td>3.95 ± 0.01</td>
<td>0</td>
<td>4.0</td>
<td>25 ± 7</td>
<td>1.0 ± 0.3</td>
</tr>
</tbody>
</table>

Analysis of Variance for % Zn Absorbed

<table>
<thead>
<tr>
<th></th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effect of protein</td>
<td>39.87</td>
<td>0.0001</td>
</tr>
<tr>
<td>Effect of flour type</td>
<td>5.02</td>
<td>0.04</td>
</tr>
<tr>
<td>Protein X flour interaction</td>
<td>0.84</td>
<td>0.4</td>
</tr>
<tr>
<td>Root Mean Square Error</td>
<td>5.4</td>
<td></td>
</tr>
</tbody>
</table>

† By analysis
∗ Mean ± SD for 10 observations
DISCUSSION

One objective of this study was to determine whether a controlled source of protein would enhance human zinc absorption. The enhancing effect of increasing amounts of casein on zinc absorption in the present study is consistent with the observations of Sandström et al. (2) that the amount of protein from various sources is positively correlated with zinc absorption. Absorption measurements in the present study are quite similar to those of Sandström et al. (2) who reported zinc absorption was enhanced from 13.2 to 15.7% with white bread, and from 8.2 to 14.0% with whole-meal bread when the amount of protein was increased from approximately 6 to 19 g with milk and cheese. It is not known whether the observations made with casein in the present study may be generalized to all sources of dietary protein. Recently, Sandström and associates (3) reported a positive relationship between zinc absorption and 15 to 44 g protein from beef, chicken, fish, and milk plus cheese. The magnitude of the enhancing effect of these various sources of protein on zinc absorption was similar to that found in the present study using casein.

Another objective of the present study was to determine whether eating more than 30 g of protein would result in further enhancement of zinc absorption, or whether maximum enhancement of zinc absorption would occur at a more moderate protein level. Sandström et al. (2) varied meal protein from 5 to 27 g with 3.5 mg zinc and more recently (3) from 15 to 44 g with variable amounts of zinc. The present study shows that additional protein up to at least 51 g enhances zinc absorption. In fact, the second addition of 20 g protein was at least as influential as the first, with no indication that a maximum level of enhancement had been reached. Maximal enhancement of zinc absorption by protein (from egg white) was observed in rat studies, and the level of protein required for maximum enhancement of absorption was dependent on the amount of zinc in the meal (5). A similar maximum enhancement might be observed in human subjects if more protein and/or less zinc were used than in the present study. Most sources of protein in human diets are also significant sources of zinc (9), and increased zinc consumption per se results in lower fractional (percent) absorption and greater absolute amounts of zinc absorbed (16,17,18). The effect of protein on zinc absorption by persons consuming different amounts of zinc, and a possible interaction between the amount of protein and zinc in a meal, needs further investigation.

A final objective of the present study was to determine whether the presence of phytate- and fiber-containing wheat bran affected the enhancement of zinc absorption by protein. Although differences between the two types of flour seemed to decrease as the protein content of the rolls increased, no significant interaction was detected between the amount of protein and the type of flour. The protein content of the meal appeared to influence zinc absorption more than the degree of extraction of the flour. The present observation that zinc absorption was lower from whole-wheat rolls than from white rolls which were supplemented to a similar zinc content is consistent with the results of Sandström et al. (2). (When zinc is not supplemented, more zinc is available from whole-wheat bread because of its higher zinc content (2).) Because of the additional zinc from sodium caseinate or zinc sulfate used in the present study to equalize zinc content, the phytic acid to zinc molar ratio of whole-wheat rolls was reduced from 22 to 11; this probably diminished the inhibiting effect of phytic acid on zinc absorption (19). An interaction between the protein level and roll type may have been observed with a higher phytic acid to zinc molar ratio; however, this would be unlikely to occur in practical diets and, experimentally, would require addition of phytic acid and/or use of a protein with unusually low zinc content, such as egg white.

The non-phytate phosphorus content of the rolls increased with each addition of sodium caseinate (Table 1). This variation was not corrected with supplemental phosphorus because non-phytate phosphorus has generally been found to have no effect (20,21,22) or a negative effect (23) on zinc retention in humans. In the present study, any possible negative influence of non-phytate phosphorus was apparently overwhelmed by the strong positive influence of protein on zinc absorption.

The enhancement of zinc absorption by concurrently ingesting protein will not necessarily improve zinc nutriture. Mahalak et al. (24) reported that a moderate increase in dietary protein (from 65 to 94 g daily) slightly increased urinary zinc excretion without significantly affecting zinc balance. Gregor and Snedeker (23) found a high protein diet was associated with greater urinary zinc, and slightly better or worse zinc retention, depending on the dietary phosphorus content. The increase in zinc absorption with higher protein intake may be balanced by a corresponding increase in excretion. A recent observation in this laboratory indicated that weanling rats required as much or more zinc for growth when protein was elevated from 15 to 30% of the diet (25).
The mechanism for enhancement of zinc absorption by dietary protein is not known. Certain amino acids and dipeptides have been reported by Wapnir et al. (26) to enhance zinc absorption by the perfused rat ileum. Those investigators found that excess L-histidine competitively inhibited absorption of an L-histidine-zinc complex by perfused ileal segments of adult rats. Possibly, amino acid or peptide ligands may bind zinc in the intestinal lumen and facilitate its absorption through carrier mechanisms that are specific for the ligand rather than for zinc. Such a hypothesis requires further testing.

In conclusion, in a study of 20 healthy adults consuming rolls labelled with $^{65}$Zn, increasing the amount of casein protein between 10 and 50 g increased zinc absorption with no indication that a maximum effect of protein had been reached. Zinc absorption from rolls with similar zinc content was greater when white flour rather than whole-wheat flour was used. The protein type and type of flour did not interact to affect zinc absorption. Zinc absorption can be substantially enhanced by the addition of protein from casein to a meal.

ACKNOWLEDGEMENTS

We are grateful to Ms. Emily Nielsen for recruitment and organization, Ms. Karen Speaker and Mr. Mitch Nelson for technical assistance with whole body counting, Ms. Bonita Hoverson and her staff for evaluation of food records, Mr. John Steiner and Ms. Sandra Gallagher and her staff for chemical analyses, and to the twenty participants who made the investigation possible.

REFERENCES


Accepted for Publication January 21, 1991